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Foliar anatomical study of *Thaumatococcus daniellii* (Benth.) Benth. and *Megaphrynium macrostachyum* (Benth) Milne-Redh. in Ile-Ife and Osogbo environs, Osun State, Nigeria

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ABSTRACT

Foliar, petiole and stem anatomical characters of *Thaumatococcus daniellii* and *Megaphrynium macrostachyum* in Ile-Ife and Osogbo, environs, Osun State in Nigeria are reported. The aim is to determine the macro and micro-characters that could facilitate their identification and classification. It is envisaged that this will further enhance the conservation of the two species and prevent them from extinction. Transverse sections of the leaf, petiole and stem were undertaken using Reichert Sledge Microtome (RSM) and observations were made using Light Microscope (LM). Diagnostic characters employable for their delimitation are their habits, stomata type, length and width of stomata, length and width of epidermal cells, anticlinal walls and stomata index on the abaxial surfaces. Others are air spaces, presence of tannins and sand crystals, abundance of sclerenchyma cells and thick cuticle in *M. macrostachyum*, presence of crystals of calcium oxalate and carbonate in *M. macrostachyum*. However, intergeneric characters between the two genera were also reported. Over all, additional diagnostic characters which have not been used for the delimitation of the two species have been reported. All these are envisaged to remove the confusion in their taxonomy and aid their conservation even when only sterile samples are available.

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Keywords: Leaf, petiole, stem, anatomy, diagnostic, conservation.

INTRODUCTION

Thaumatococcus daniellii (Benth.) Benth the sweet prayers plant which is otherwise known as “ewe eran” by the Yoruba people of Southwest Nigeria or “Katemfe” in Sierra Leone is a perennial, rhizomatous and monocotyledonous herb that grows through its rhizomes (Jennings et al., 2001). It belongs to the family Marantaceae along with *Megaphrynium macrostachyum* (Benth)

Milne-Redh with which it shares similar morphological and ethnobotanical usage (Jennings et al., 2001). As a result of their morphology and ethnobotanical uses, there are a lot of controversies among field taxonomists and local community people that utilizes these two plant species. While *Thaumatococcus* has only one species (*T. daniellii*) in whole of West Africa, Nigeria inclusive (Hutchinson and Dalziel, 1963), *Megaphrynium* has two

species but interestingly *M. macrostachyum* is the only species that can be found in Nigeria (Hutchinson and Dalziel, 1963). They are both indigenous to the rainforest of West Africa particularly the Southern parts of Ghana, Cote d'Ivoire and Nigeria (Jennings et al., 2001; Arowosoge and Popoola, 2006).

Ethnobotanically, *Thaumatococcus daniellii* is known to have multipurpose usefulness among local people, ranging from cultivation as fetish plant in Gabon to wrapping and boiling food with the leaves in Ghana as well as in Nigeria (Ojekale et al., 2007). The stalks are popular for weaving mats, baskets, fishnets and other valuable resources. Lately the stalk has been discovered to be a potential for pulp and papermaking while its roots for curing many ailments (Oluwadare and Sotande, 2005, 2006; Ojekale et al., 2007). At maturity, the plant produces flowers, which later develop into an edible berry containing high amount of Thaumatin (Oluwadare and Sotande, 2005). The sweetener can be used in pharmaceutical "mineral drinks", beverages and confectionery industries (Ojekale et al., 2007).

Megaphrynium macrostachyum equally have similar ethonobotanical usage as *Thaumatococcus daniellii*. The leaves are also used in wrapping food in order to preserve the food (White, 2001). In Central Africa the vegetative parts are an important food of Gorillas, Chimpanzees and Baboons (Jiofack, 2011). The leaves have fibers that are very useful in roof thatching as well as making cushion for sleeping mats (Terashima and Ichikawa, 2003). There are other numerous examples of disposable utensils made of *Megaphrynium macrostachyum*, for example the leaves are used as instant containers for carrying forest products, substitute for pots,

cooking sheets, plates, cups, and funnels (Hattori, 2006). *Megaphrynium macrostachyum* leaves are used to supply water to catch fish in the water pools, as eyedroppers, fans to build a fire, dust pans, parasols for a baby and tobacco rollers (Hattori, 2006). It can be used as an antidote against arthropod or snake poisoning and venoms (Okungbowa et al., 2013).

Over exploitation of both *Thaumatococcus daniellii* and *Megaphrynium macrostachyum* has affected their populations negatively and if this continues, might results in their extinction. And again, confusion in their taxonomy has not helped in their cultivation. The problems associated with the inability to cultivate these species have led to the use of nylon for food wrapping by some food vendors.

Anatomical characteristics have continued to be useful as a tool for species classification and identification, most especially when only sterile samples are available (Bieras and Sajo, 2004; Passos et al., 2009). There was no study focused on the usefulness of Maranthaceae plants (Hattori, 2006). Moreover, detailed anatomical study has not been done on *Thaumatococcus daniellii* and *Megaphrynium macrostachyum*. The aim of this study therefore is to determine the macro and micro anatomical characters that could be used their identification and classification. It also attempts to create the awareness on the need to conserve these economic plants because some of them are becoming rare species in our environment.

MATERIALS AND METHODS

Study area and sample collection

Mature stems and leaves of *Thaumatococcus daniellii* were collected fresh

from the Biological garden of Obafemi Awolowo University, Ile-Ife, Nigeria (7°51'N 4°52'E). While the mature leaves of *Megaphrynium macrostachyum* were collected from two villages namely, Tonkere in Ile-Ife (7°616'N 4°516'E) and Aratumi in Atakumosa West Local Government area (7°525'N 4°670'E), Osun state, Nigeria. The specimens were identified by a specialist and herborized with a voucher specimen of each plant being deposited at the Herbarium of the Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria (IFE).

Leaf, petiole and stem anatomical study

The leaves of each species were cut into sizeable portions and were macerated inside concentrated nitric acid in plastic petri dishes (Folorunso, 2011). Epidermal peels of both adaxial and abaxial surfaces were separated and stained in 1% ethanol solution of Safranin O for 5 minutes; they were rinsed carefully in water to remove excess stain and mounted in 25% glycerol. Twenty slides from each of the upper and the lower epidermis were prepared per slide and studied. The stomata index (S.I.) was also calculated as the percentage proportion of the number of stomata to the other epidermal cells present on leaf portion (Dilcher, 1974). This was done for both adaxial and abaxial surfaces of the leaves using the formula.

$$\text{Stomata Index (S.I.)} = S / (E+S) \times 100$$

Where;

S= Number of stomata per unit area.

E= Number of epidermal cells in the same unit area.

These measurements were then converted into micrometer by multiplying each of the measurement by the ocular constant with respect to the power under

which they were taken. Transverse sections of the petiole, leaf and stem were cut at 20 μm thickness with the aid of Reichert Sledge microtome. The sections were stained in 1% ethanol solution of Safranin O for 5 minutes; they were washed in 3 changes of water, dehydrated through series of ethyl alcohol percentages and mounted in 25% glycerol. All measurements were made at $\times 40$ objective lens with the aid of ocular and stage micrometer. Photomicrographs of the slides were made with an Accu-scope trinocular microscope (ACCU-scope 3001 LED Trinocular microscope with 3.2 MP CMOS digital camera).

RESULTS

Leaf epidermis

The habits of *T. daniellii* and *M. macrostachyum* are as shown in Figures 1a and b respectively. There is a dark green region along the mid-rib of all *M. macrostachyum* studied and again they have big and robust size when compared with *T. daniellii*. The stomata on the abaxial surface of *T. daniellii* are paracytic (Figure 2a) and tetracytic in *M. macrostachyum* (Figure 2b). The adaxial surface of *T. daniellii* also has paracytic stomata (Figure 2c) while that of *M. macrostachyum* has hexacytic stomata (Figure 2d). The epidermal cell walls are thick in the two plants. Shapes of epidermal cell walls are irregular in the two plants (Figure 2). The anticlinal walls are wavy in *T. daniellii* but are more or less straight in *M. macrostachyum* (Figure 2a-d). On the adaxial surface for *T. daniellii*, the length of stomata measured 3.29 μm -3.66 μm long, average length was 3.47 μm , width of stomata ranges from 2.19 μm -2.56 μm , average width was 2.37 μm ; for *M. macrostachyum*, stomata length measured

2.19 μm -3.66 μm , average length 3.11 μm , width of stomata measured 2.19 μm -2.92 μm , while the average width is 2.45 μm (Table 1). The frequency of stomata per unit area was 0-2 and the average is 1 in *T. daniellii*; for *M. macrostachyum*, it ranges 0-1 with an average of 1. The epidermal cells frequency for *T. daniellii* range from 142-271 with average of 185, in *M. macrostachyum*, the epidermal frequency ranges from 156-231 with an average of 183 (Table 1). Stomata index was 0.53% in *T. daniellii*; 0.53% in *M. macrostachyum* (Table 1). The length of epidermal cell for *T. daniellii* is from 2.92 μm -4.39 μm with an average of 3.66 μm ; for *M. macrostachyum*, epidermal cell length ranges from 3.66 μm -7.68 μm and an average of 6.40 μm . The width of epidermal cells for *T. daniellii* ranges from 1.09 μm -2.19 μm with an average of 1.57 μm ; for *M. macrostachyum*, it is 1.46 μm -2.92 μm and an average of 2.04 μm .

On the abaxial surface for *T. daniellii*, the length of stomata measured 1.83 μm -2.56 μm long, average length was 2.12 μm , width of stomata ranges from 1.09 μm -1.83 μm , average width was 1.56 μm ; for *M. macrostachyum*, stomata length measured 1.83 μm -2.92 μm , average length 2.41 μm , width measured 1.83 μm -2.56 μm , average width 2.08 μm (Table 1). The frequency of stomata per unit area was 53-64 and the average is 57 in *T. daniellii*; for *M. macrostachyum*, it ranges 42-60 with an average of 52. The epidermal cells frequency for *T. daniellii* range from 301-383 with average of 328, in *M. macrostachyum*, the epidermal frequency ranges from 205-271 with an average of 250 (Table 1). Stomata index was 14.7% in *T. daniellii*; 16.9% in *M. macrostachyum* (Table 1). The epidermal cell

length for *T. daniellii* measured from 1.83 μm -4.02 μm with an average of 3.07 μm ; for *M. macrostachyum*, it is 2.92 μm -4.02 μm and an average of 3.51 μm . The width of epidermal cells for *T. daniellii* ranges from 1.09 μm -2.19 μm with an average of 1.75 μm ; for *M. macrostachyum*, it is 1.83 μm -3.66 μm and an average of 2.74 μm (Table 1).

Petiole anatomy

The petiole outline is round with 5-7 air spaces in *T. daniellii*, the outline is also round in *M. macrostachyum* but with 7-9 air spaces (Figure 2e and f). In these two species of Maranthaceae, the vascular bundles are scattered in the ground tissue. Each vascular bundle consists of a lysinoginous cell (water storing cavity). The wall of the ground parenchyma cells contains calcium oxalate crystals (Figure 2e and f). Again in both species, towards the epidermis are many patches of sclerenchyma cells which completely surround the vascular bundle. There are tannins and sand crystals in *T. daniellii* (Figure 2e and f).

Transverse sections of leaf

In both species, the palisade cells are towards the upper surfaces, they are rectangular and elongated. The vascular bundles arranged in parallel series are collateral and closed (Figure 3a and b). There are more sclerenchyma cells on both ends of the vascular bundle in *M. macrostachyum* than in *T. daniellii*. The number of vascular bundles scattered in the mid-rib of the two species are more or less the same. In *T. daniellii*, a long uniseriate and unicellular hair projects from the vascular bundle to the outside (Figure 3a and b).

Transverse sections of stem

In *T. daniellii* (Figure 3c), the epidermis with uniseriate multicellular hairs is surrounded by a thin layer of cuticle. One to three layers of collenchyma cells which are rectangular or polygonal are present. There are many crystals of calcium oxalate in form of bundles of raphides, prismatic crystals which are rhomboidal in shape are present. Some spiral shaped crystals are also present and there are oil ducts. In the ground tissue are few sclerenchymatous cells, lysinoginuous cavity are also present. In the tangential longitudinal section (Figure 3e), the vessels are largely spiral.

In *M. macrostachyum* (Figure 3d), the epidermis with a 3 layers of very small polygonal cells is surrounded by a thick layer

of cuticle. Below the epidermis are two layers of sclerenchymatous hypodermal cells which are polygonal. There is one to two layers of chlorophyllous tissue beside which are many patches of sclerenchymatous cells that completely surrounded the vascular bundles. The vascular bundles which are closed and collateral are scattered in the ground tissue. They are incompletely surrounded by a sheath of sclerenchyma with a distinct portion on the outer side in form of a cap. There are crystals of calcium carbonate in form of cystoliths in the ground tissue. There are also crystals of calcium oxalate like raphides, idioblasts, druses and sand crystals. In the tangential longitudinal section (Figure 3f), xylem vessels are annular.

Table 1: Abaxial and adaxial epidermal features of *T. daniellii* and *M. macrostachyum*.

Features	<i>T. daniellii</i>		<i>M. macrostachyum</i>	
	Abaxial	Adaxial	Abaxial	Adaxial
Stomata type	Paracytic	Paracytic	Tetracytic	Hexacytic
Length of stomata (μm)	2.12 ± 0.21	3.47 ± 0.00	2.41 ± 0.29	3.11 ± 0.49
Width of stomata (μm)	1.56 ± 0.19	2.37 ± 0.18	2.08 ± 0.23	2.45 ± 0.28
Frequency of stomata per unit area	53 – 64 (57)	0-2 (1)	42 – 60 (52)	0-1 (1)
Shape of epidermal cells	Irregular	Irregular	Irregular	Irregular
Length of epidermal cells (μ)	3.07 ± 0.99	1.57 ± 0.51	3.51 ± 0.54	6.40 ± 1.16
Width of epidermal cells (μ)	1.75 ± 0.31	1.57 ± 0.32	2.74 ± 0.65	2.04 ± 0.48
Frequency of epidermal cells per unit area	301 – 383 (328)	142-271 (185)	205 -271 (250)	156-231 (183)
Stomatal index	14.70%	0.53%	16.90%	0.53%
Trichomes	Non-glandular uniseriate	Absent	Clublike	Absent
Epidermal cell wall	Thick	Thick	Thick	Thick
Tannins	Present		Present	Present

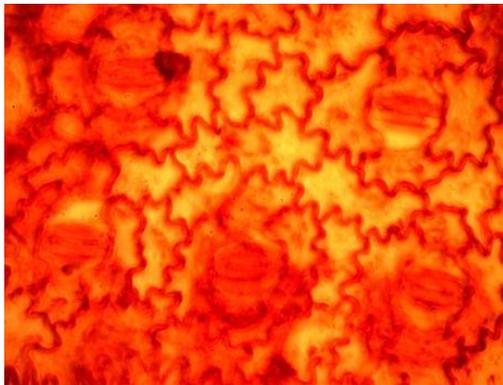


Figure 1 (a)

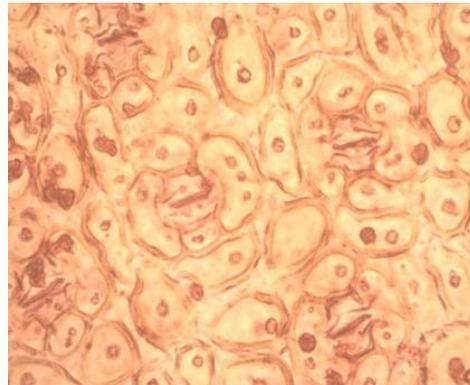


Figure 1 (b)

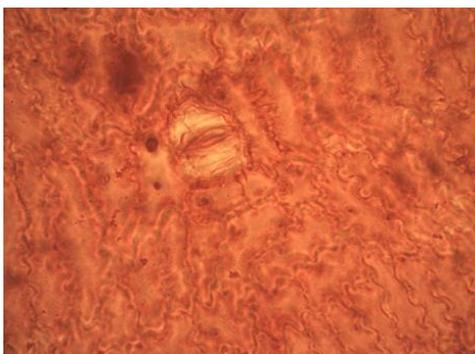
Figure 1: Habits of *T. daniellii* and *M. macrostachyum* respectively. Scale = 30 μ m.



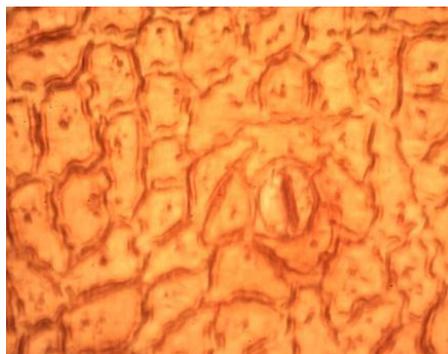
(a) *T. daniellii* abaxial



(b) *M. macrostachyum* abaxial



(c) *T. daniellii* adaxial



(d) *M. macrostachyum* adaxial

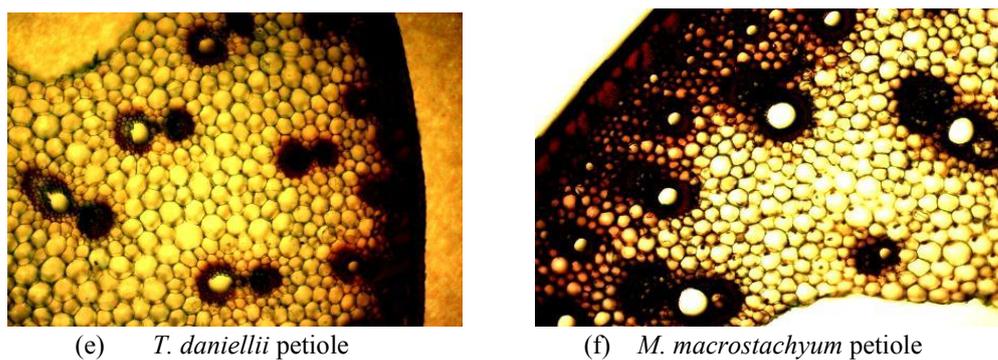


Figure 2: Epidermal and petiole anatomy of *T. daniellii* and *M. macrostachyum*. Scale = 25 μ m.

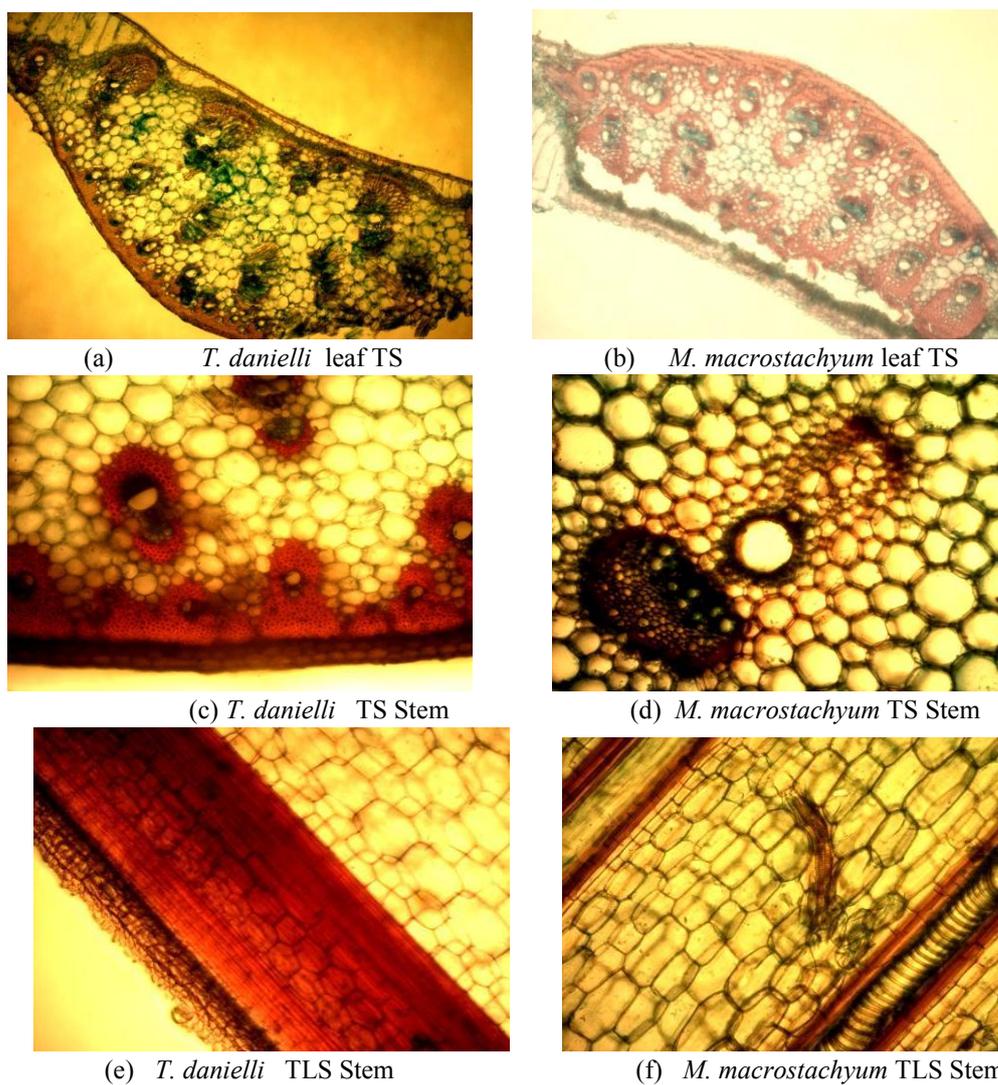


Figure 3: Sections of Leaf and Stem of both *T. daniellii* and *M. macrostachyum*. Scale = 25 μ m.

DISCUSSION

The diagnostic characters in the foliar epidermis which delimit these two species of Maranthaceae are their habits, stomata type, length and width of stomata, length and width of epidermal cells, anticlinal walls and stomata index on the abaxial surfaces. Several workers have reported the usefulness of foliar epidermal characters in the delimitation of species (Jayeola et al., 2001; Adedeji and Illoh, 2004; Ogundare and Saheed, 2012; Folorunso et al., 2014). The two species can be separated using their macro-morphological characters. The large and robust size together with the dark green mid-rib area conveniently delimits *M. macrostachyum* from *T. daniellii*. The presence of tetracytic and hexacytic stomata in *M. macrostachyum* clearly delimits it from *T. daniellii* which has just paracytic stomata. This may also contribute to the high stomata index on the abaxial surface of *M. macrostachyum*.

Looking at the petiole anatomy, *M. macrostachyum* has more air spaces than *T. daniellii*. The presence of tannins and sand crystals in *T. daniellii* is noteworthy. Tannins are antioxidants and prevent the onset of degenerative diseases such as cancer and cardiovascular diseases (Zhang et al., 2014). In the leaf transverse sections, the sclerenchyma cells which are abundant in *M. macrostachyum* might have contributed to the large and robust sizes earlier reported in their habits. This also might be the reason why they wrap more food than *T. daniellii*. However, *T. daniellii* is more widely known and more often used for food wrapping than *M. macrostachyum*. The presence of sweet, non-toxic and heat stable protein called Thaumatin in *T. daniellii* have been reported (Oluwadare and Sotande, 2006). This protein has been used as natural sweetener and employed as taste modifier in various foods and beverages production. The long uniseriate and unicellular hairs reported for *T. daniellii* might

have played a major role in the production of this Thaumatin.

The anatomical architectural designs in the stem of these two species are quite different. The thick layers of cuticle, abundance of sclerenchymatous cells which completely surround the vascular bundles in *M. macrostachyum* delimit it from *T. daniellii*. Plants need mechanisms of defense to limit the amount of damage by herbivores; a plant with poor defenses faces the possibility of severe foliage damage, rendering it incapable of performing an adequate amount of physiological or reproductive measures to survive (Doerge, 2003). Among these mechanisms are the productions of calcium oxalate and calcium carbonate crystals. In *T. daniellii*, crystals of calcium oxalate either in prismatic shape or raphide form are present whereas in *M. macrostachyum* both crystals of calcium oxalates and carbonates are reported. Zhang et al. (2014) reported how they used calcium oxalate crystals for the identification of decayed tea plants (*Camellia sinensis* L.). *T. daniellii* is widely used to wrap almost all food materials; there is limitation to the use of *M. macrostachyum*. For example, the leaf of *M. macrostachyum* is not used to wrap certain food items such as food product from bean seed (Moinmoin) because it will alter the colour of the products turning it black and making the food detestable to consumers. However, it is used in wrapping solid food items such as pounded yam because of its thickness and ability to preserve food for long. In spite of this limited usage, our data suggests that *M. macrostachyum* is more threatened as a result of over exploitation. This observation was earlier reported by Jiofack (2011), a possible explanation for this over exploitation might be because of its usefulness in herbal remedies, making roof thatching, forest products, and as substitute for pots, cooking sheets, plates, cups and funnels (Terashima and Ichikawa, 2003).

Conclusion

This study has provided the macro and micro anatomical characters that could be used for the identification and classification of both *T. daniellii* and *M. macrostachyum*. Additional diagnostic characters which have not been used for the delimitation of the two species have been reported. *T. daniellii* is widely used to wrap almost all food materials whereas there is limitation to the use of *M. macrostachyum*. *M. macrostachyum* is more threatened by over exploitation as it is usually collected from the wild away from houses.

COMPETING INTERESTS

The authors declared that they have no competing interests.

AUTHORS' CONTRIBUTIONS

The corresponding author conceived the idea, designed the work and supervised the last author during the project. The remaining two authors contributed in the data collection and analysis of the project.

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